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Metallomic Distribution in Various Regions of the Brain as Influenced by Dietary Intakes and Their Implications

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Abstract.

Lifelong exposure to environmental factors can influence the risk of developing diseases according to recent research findings. Environmental stresses ultimately leading to neuronal cell death have been hypothesized as the causes of the increased occurrence in developing Alzheimer's and Parkinson's disease. Our daily diet is considered to be one of the most important environmental factors that can seriously affect the development and proper functions of the brain. Depending on the concentrations, metals and electrolytes can post some health concerns, especially for a prolonged consumption period. For example, it was reported that excess amounts of iron, zinc and copper in the human brain may cause oxidative damage and protein aggregation; the neurotoxicity induced by these metals may lead to cerebral and/or cerebellar degeneration. Other reports showed that there were differences in concentrations of five different elements (aluminum, zinc, copper, manganese, and iron) between normal human brain and brains of patients with Alzheimer's disease. In this study, we investigated 30 elements, including electrolytes, and how dietary intake on a life-time basis would affect their concentrations and distributions in various regions of the rat brain (hypothalamus, cerebellum, pons and medulla, striatum, mid-brain, cerebral cortex, and hippocampus) and discussed their health implications. Information matrices of these 30 different elements (mostly metals) and their distributions in various regions of the rat brain were analyzed as a function of normal dietary intake at different ages during development. Our results showed that metallomic distribution in various regions of the rat brain is age-related. The results may help researchers to identify possible links between daily dietary intake of metals and electrolytes and diseases associated with aging (e.g., Alzheimer's and Parkinson's disease) and suggest such metallomic distributions may be used as neurological biomarkers of exposure to heavy metals.

Key words: Brain; elements; metals; electrolytes; distribution; metallomic; neurological; diseases

1. Introduction

Many of the metals around us are essential for life, but others are known to be highly toxic; even the essential metals can be toxic when their intakes are too high. For example, metals such as chromium, cobalt, copper,

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manganese, and zinc are essential for life. But, an excess intake of these essential metals can induce toxicity. Some metals such as arsenic, cadmium, lead, mercury, and vanadium, which are found throughout our environment, are toxic to humans and other animals. Some of these toxic metals are even capable of forming covalent bonds with carbon, resulting in metal-organic transformations. This type of transformation affects the mobility and toxicity of the element. Elements can come from naturally-occurring processes such as volcanoes, water, bacterial activity, and also from anthropogenic sources such as automobile exhaust, agricultural fertilizers, industrial activities, and many other sources. It has been shown that in highly industrialized areas, the exposure to metallic elements is extremely high [1].

Being a specialized organ of the body, the brain metabolizes and accumulates metals as part of its normal development and function. But in a rich metal environment, loss of metalloproteins and loss of defense against oxidative stress caused by one or more of the heavy metals could be responsible for neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD) [2]. The intake of these metals occurs via ingestion of metal-containing food and water, and/or through inhaling metal-contaminated air. The elemental distributions in the different brain regions appear to vary for each element. Some of these metallic elements are known to increase or decrease in brains of humans with a neurodegenerative disorder.

The following are some of the more studied elements in brain; other elements are also found in brain, but research on the latter group has been scant. Although many metals are normally found in brain, over-accumulation of metals may lead to health problems. Moreover, an unbalanced increase or decrease could cause a major functional change and lead to a neurodegenerative disorder even though the underlying molecular mechanisms are largely unknown.

Aluminum

Aluminum (Al) is one of the more widely distributed metals in the environment. Approximately 8% of the earth's crust is Al. The exposure of Al normally occurs through air, food, and water [3]. The brain contains approximately 1% of the body's total Al; however, Al has no known function in normal mammalian brain.

During the life-span of a normal human brain, its Al levels appear to increase around age 40, and then plateau at about age 70. The Al levels then begin to increase again from age 80 to 90. The globus pallidus (GP), substantia nigra (SN) and the nucleus ruber appear to be the highest Al levels in the normal human brain [4]. The levels of Al increase in the grey matter in patients with dialysis encephalopathy [5]. Also, Al accumulations in the brain have been found to increase in patients with renal failure. Following oral exposure to this metal, retention of Al was reported in the hippocampus. This region of the brain is rich in cholinergic neurons. These and other observations suggest that Al has neurotoxic properties. Furthermore, following chronic exposure, Al has been shown to accumulate in all regions of the rat brain. There is evidence of a relationship between the high levels of Al and increased risk of neurodegenerative disorders, including AD and PD [3].

Calcium

Calcium (Ca) is the fifth most abundant element found in the earth's crust. Ca occurs most commonly in sedimentary rocks in the minerals calcite, dolomite, and gypsum. Ca is essential for life in most living organisms, including humans. Ca has been shown to be elevated in the brains of patients with AD: it concentrates in the amyloid plaques. These plaques are one of the neuropathological hallmarks of AD. However, PSAPP mice (a mouse model of AD) appear to accumulate less Ca in their plaques compared to their surrounding brain tissue [6].

Chromium

Chromium (Cr) is also found in the earth's crust and is mined as chromite ore. Because of chromium's high corrosion resistance and hardness, it is added to nickel to create stainless steel: this is the most common application of Cr. It is also used in dyes and pigments, as a gasoline additive, and in the tanning of leather. The pathophysiological role of Cr has not been elucidated. However, Cr has been shown to moderately increase in the parietal cortex of patients with AD when compared to that in normal brain [7].

Copper

Copper (Cu), like most elements, is mined from the earth's crust. Furthermore, Cu is recycled. Cu is commonly used in electrical applications, piping, many household products (e.g., sinks, plumbing, pots), architectural applications, coin-making, and chemical applications. Cu has been found to be vital in human and plant life. Cu is introduced into the body mostly via food intake. Some foods that are high in Cu are oysters, beef or lamb liver, Brazil nuts, cocoa, black pepper, lobster, sunflower seeds, green olives, avocados, and wheat bran. The brain contains approximately 7.3% of the total body Cu supply.

Cu has been an extensively studied metal in the brain [8]. In a normal brain, Cu is distributed in similar ways in the central and subcortical white matter of the cerebellum of both young and old brains; however, the periphery of

the dentate nucleus is rich in Cu [2]. The higher Cu concentrations are mostly found in the cortex and hippocampus [8]. A normal aging brain has been shown to have increased Cu levels, especially in the substantia nigra and in some cerebellar regions [4]. However, when the levels of Cu in the cortex and hippocampus increase, Wilson's disease is said to result [9]. In AD, there is an abnormal brain Cu distribution, with large amounts in the amyloid plaques but a deficiency in the neighboring brain tissue. Other researchers also reported Cu to increase in the senile plaques [4]. These findings suggest the involvement of Cu in AD is multifactorial and complex.

Iron

Iron (Fe) is one of the most common metals found in everyday use. Fe makes up about 5% of the earth's crust. The earth's core is believed to consist largely of an iron-nickel alloy constituting 35% of the mass of the earth as a whole. Because of this distribution, Fe is thought to be the most abundant element on earth. Some popular food stuffs rich in Fe include red meat, fish, poultry, beans, vegetables, black-eye peas, wheat, and cereals.

Fe is another extensively studied metal in brain. Fe is necessary for normal brain function (e.g., in learning and memory) [10]. In a normal brain, Fe is distributed in similar ways in central and subcortical white matter of the cerebellum of both young and old brains; in the cerebellar cortex, there are high Fe levels, and the periphery of the dentate nucleus is also rich in Fe [2]. Fe appears to increase rapidly in the young brain and then remains stable until the later years; increases in Fe in the substantia nigra and globus pallidus have been reported [4]. Regions where brain Fe is high include substantia nigra, globus pallidus, red nucleus, caudate nucleus, and the putamen [11].

Like Cu and Ca, Fe has been shown to be elevated in brains of patients with AD. Elevated Fe is found in the amyloid plaques [6]. Fe is also moderately increased in the parietal cortex in AD patients [7]. In patients with Attention Deficit Hyperactivity Disorder (ADHD), Fe has been shown to increase in the substantia nigra [12].

Manganese

Manganese (Mn) is found in the earth's crust and in seawater. It is often found with Fe. Some of the more common usages of Mn are in steel, aluminum alloys, alkaline batteries, coins, and pigments. Some popular foods that contain Mn include tea, spinach, grains, rice, eggs, nuts, olive oil, green beans, and fish.

The majority of the human body's Mn is found in liver and kidneys; nevertheless, Mn is an important element for normal brain development and function [13]. In the aging brain, Mn has been shown to redistribute itself in different brain regions, including hypothalamus, thalamus, and corpus callosum [4]. High brain Mn concentrations are related to PD. There has been much research indicating that human striatum, globus pallidus, and substantia nigra show increases in Mn levels and are thought to be target sites for Mn neurotoxicity. The globus pallidus and pituitary glands are other regions where Mn preferentially accumulates. High exposures to Mn result in increases in Mn in the olfactory epithelium and olfactory bulb [14]. It is noteworthy that Mn levels in the cerebellum are similar in AD and normal brains while Mn increases in the parietal cortex of AD brains [7].

Magnesium

Magnesium (Mg) is the 8th most abundant element in the earth's crust and in found also in seawater. Mg is commonly used in structural building materials, automotive parts, electronic devices, aerospace construction, photography, and fireworks. Mg is an essential metal in human and plant life [13]. Human Mg deficiency has been linked to the development of asthma, ADHD, and osteoporosis. Foods that are high in Mg include spices, nuts, cereals, coffee, cocoa, tea, and vegetables. In neurodegenerative disorders such as AD and PD, Mg does not appear to play a major role. For example, Mg levels in the cerebellum and parietal cortex do not differ between patients with AD and normal humans [7].

Mercury

Mercury (Hg) is found in deposits throughout the world, mostly as cinnabar (a common ore of Hg). The more common uses of Hg are in barometers, thermometers, dental products (e.g., fillings), and electrical equipment (e.g., computers, telephones, etc.) [15]. Exposures to Hg most commonly occur from the consumption of marine species [1]. Other exposures to Hg may be from exhaust of coal-burning power plants, cement production, batteries, and gold production. The role of Hg in neurological diseases such as AD and PD has been controversial. Hg can cross the blood-brain and blood-placental barriers. It is then retained by the brain for years [16]. Hg tends to accumulate in lipid-rich regions of the brain. Human exposure to Hg usually results in kidney and neurological disorders [15].

Potassium & Rubidium

Potassium (K) occurs in nature as an ionic salt and is essential for humans and other animals. It is found dissolved in seawater and in other minerals. Some common food sources of K include orange juice, potatoes, bananas, avocados, tomatoes, broccoli, apricots, and many other fruits.

Rubidium (Rb) is not essential for humans, but is readily taken up by the body. It is found commonly mixed in with other elements, especially K. Rb is found in some plants as well. Some common uses of Rb are in fireworks, lasers, chemical applications, and electronic transmission. Rb is found in come brain tumors.

Exposure to K and Rb is not known to cause neurodegenerative disorders. For example, the concentrations of K and Rb are similar between AD and control brains [1; 7].

Sodium

Sodium (Na) is an essential element for humans and other animals. It is found in nature as a compound only; the most common forms are salt deposits. The most common form of Na that we know and use is sodium chloride, table salt. It does have many other industrial uses as well. Over- or under-exposure to Na has not been shown to cause neurological disorders. Nevertheless, Na has been shown to increase every brain region in AD patients [1].

Zinc

Zinc (Zn) is naturally found in the earth's crust and in seawater. It is normally found with Fe and Cu deposits. Zn is commonly used in batteries, production of brass, bronze, rubber, in pigments, fire retardant, nuclear weapons, automobile engines, and agricultural fungicides. The most common food source of Zn is red meats.

Zn is another essential element in mammals. Zn is distributed in similar ways in the central and subcortical white matter of both young and old brains; the interior of the dentate nucleus and the cerebellar cortex contain high levels of Zn [2]. The regions of the normal brain rich in Zn include the hippocampus, amygdala, and the cortex. As the brain ages, its Zn distribution changes, just in the regions mentioned above. Nevertheless, little or no decrease in brain Zn has been reported in aging humans or rats [4]. Zn is found to be elevated in human amyloid plaques while PSAPP mice (an AD mouse model that does not show neurodegeneration) only had a 29% increase of Zn in their plaques compared to the Zn in brain tissue surrounding the plaques [6]. The potential role of Zn as a cofactor in the pathogenesis of AD was strengthened when Zn enrichment was found in senile plaques and a Zn elevation in the neuropil of AD patients as compared to those in control individuals [4].

Of the 90 plus naturally-occurring elements, 26 are known to be essential for humans and animals [17]. These consist of 11 major elements or macro-elements. They are: C, H, O, N, S, Ca, P, K, Na, Cl, and Mg. Fifteen elements are known as trace elements or micro-nutrients. They include: Fe, Zn, Cu, Mn, Ni, Co, Mo, Se, Cr, I, F, Sn, Si, V, and As. The molecular bases for the essential element selection and rejection have not been elucidated, however. Major elements, such as Na, K, Ca, and Mg, are required for bodily functions such as body fluid buffer, active transport, ionic balance, electrical transmission, tissue development as well as the composition of body fluids and structures. Trace elements act primarily as catalyst in enzyme systems in cells where they serve a wide range of functions from weak ionic effects to highly specific associations such as metalloenzymes. In addition, the protein-metal interactions may increase the stability of the protein moiety to metabolic turnover.

In this study, we investigated 30 elements (including electrolytes) and how dietary intake on a life-time basis would affect their concentration and distribution in various regions of the rat brain (hypothalamus, cerebellum, pons and medulla, striatum, mid-brain, cerebral cortex, and hippocampus) and discussed their health implications. The 30 elements of interests were: Al, As, Ba, Br, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, Hg, I, K, La, Mg, Mn, Mo, Na, Rb, S, Sb, Sc, Se, Si, Sm, Sr, V, and Zn. In the past, research has primarily focused on the effects of different elements on the brain and the neurological disorders they may cause. None of those studies, however, have examined simultaneously as many elements in a single study as we have presented here. The objective of this paper is to characterize the levels of major and selected trace elements in various regions of the rat brain during several critical stages of development. Because during the first 8-9 days of age postnatal, the rat hippocampus is extremely difficult to visually distinguish from the overlying cerebral cortex, we had included the hippocampus tissue together with the cerebral cortex for analysis in the case of 5-day-old tissue samples. The four postnatal age groups of rats we have studied include the following:

I. 5 days old;
II. 10 days old;
III. 22/23 days old; and
IV. 120 days old (i.e., adult).

While humans and rats are not identical neurologically and physiologically, there are more similarities between the two species as far as brain structure and functions are concerned. The four stages of the postnatal development of the rat chosen in this study closely parallel the postnatal development of humans. As our well being is largely impacted by the environment we are living in, dietary intake is definitely a major factor that posts a long-term effect on our health. This paper provides some insight into how our diet and pollutants in our diet may affect our neurological health. Consequently, our experimental results may allow us to take some preventative measures in

determining how best to optimize and gauge our dietary intake of major and trace elements for the betterment of our health.

2. Experimental Methods

Wistar rats of the Portion strain (Animal Breeding Unit, Carshalton, Surrey, U.K.) bred in the Institute of Neurology, University of London, U.K. were used. All animals were kept on a 12-hour light/12-hour dark cycle with free access to food and water. Elemental concentrations in the rat food pellets had been determined (Table 1 below).

Table 1. Elemental Concentration of rat food pellet.

Element	Concentration (in mg/g)
Ca	6.39 ± 1.68
Cl	2.31 ± 0.49
Fe	0.25 ± 0.05
K	5.36 ± 0.95
Mg	1.17 ± 0.32
Na	1.48 ± 0.32
	Concentration (in μg/g)
Al	98.06 ± 8.05
Br	10.57 ± 2.26
Co	0.20 ± 0.04
Cr	1.50 ± 0.44
Cu	7.21 ± 2.12
F	ND(5.00)
Hg	ND (0.25)
I	ND (0.50)
Mn	49.34 ± 8.65
Mo	2.80 ± 0.45
Se	ND (0.25)
Rb	12.73 ± 2.61
V	ND (0.50)
Zn	47.73 ± 4.11

Thirty trace and major elements in different brain regions of rats at the four different age groups (i.e., I through IV as defined above) were analyzed by instrumental neutron activation analysis (INAA) [18]. The samples were irradiated by thermal neutrons using three different sets of conditions depending on the nuclear characteristics of the elements of interest [18]. Not all 30 elements were reported in the results because those not reported herein were either below the detection limits of our INAA technique [18] or the elements were not retained by the brain tissue. All values listed are mean ± S.D.; ND = not detectable, and values in brackets are the maximum elemental concentrations present in the diet.

As already noted above, in age group I, the hippocampal tissue had been included in the cerebral cortex and analyzed as such.

All the standard materials and samples for INAA were freeze-dried and pelleted. Dried samples (rat brain regions) were homogenized using a Glen Creston polystyrene ball-mill and pelleted. Other details of standard and sample preparations were as described previously [18; 19].

Standards and samples were irradiated in a 100 KW 'Consort' Reactor Mark II at the Imperial College Reactor Centre. Irradiated standards and samples were then analyzed for their elemental contents by gamma ray spectrometry using various Ge(Li) detectors and ND6600 Multichannel Analyzer (Nuclear Data Inc., Schaumburg, Illinois, USA). Details of the elemental determinations were described in the methodology paper by Chan et al. (1983).

All laboratory chemicals used were of analytical grade (BDH Chemicals Limited, U.K.) and single element standards of trace elements were obtained from AAS (Ventron Division, Limited, U.K.); other elemental standards for F, Cl, Br, and P were obtained from Hopkin and Williams Chemical Ltd., U.K. Water for solution preparations and feeding was double-distilled.

3. Results

Cerebellum

The cerebellum is the "cauliflower-shaped" region of the brain located in the lower part of the brain next to the brain stem. The cerebellum controls movement, balance, and coordination. More recent evidence suggests that it plays roles in regulations of emotions and memory and learning. Figure 1 shows the elemental distributions in the cerebellum during rat postnatal development.

It can be noted from Figure 1 that the levels of different elements generally show a decreasing trend as the cerebellum develops and reaches adulthood: this trend may reflect the gradual closing of the blood-brain barrier between age 10 days through weanling and beyond. However, research on elemental distributions in cerebellum during development has been minimal: some of the more studied elements include magnesium, rubidium, and sodium.

Magnesium levels in the parietal cortex in AD patients do not differ from those in normal controls [7]. Similarly, rubidium levels in AD brains do not differ from those in control brains [1]. Consequently, the age-related changes in magnesium and rubidium levels in the cerebellum may not be have good predictive value of neurological diseases in later life. On the other hand, the sodium levels in the rat cerebellum decreases during the latter half of postnatal development as they approach the adult level. On the other hand, sodium levels in every regions of the human brain are significantly higher in the AD patients than those in control subjects [1]. These results taken together suggest that dietary intake of sodium may have some relevance in the causation and/or progression of AD.

Cerebral Cortex

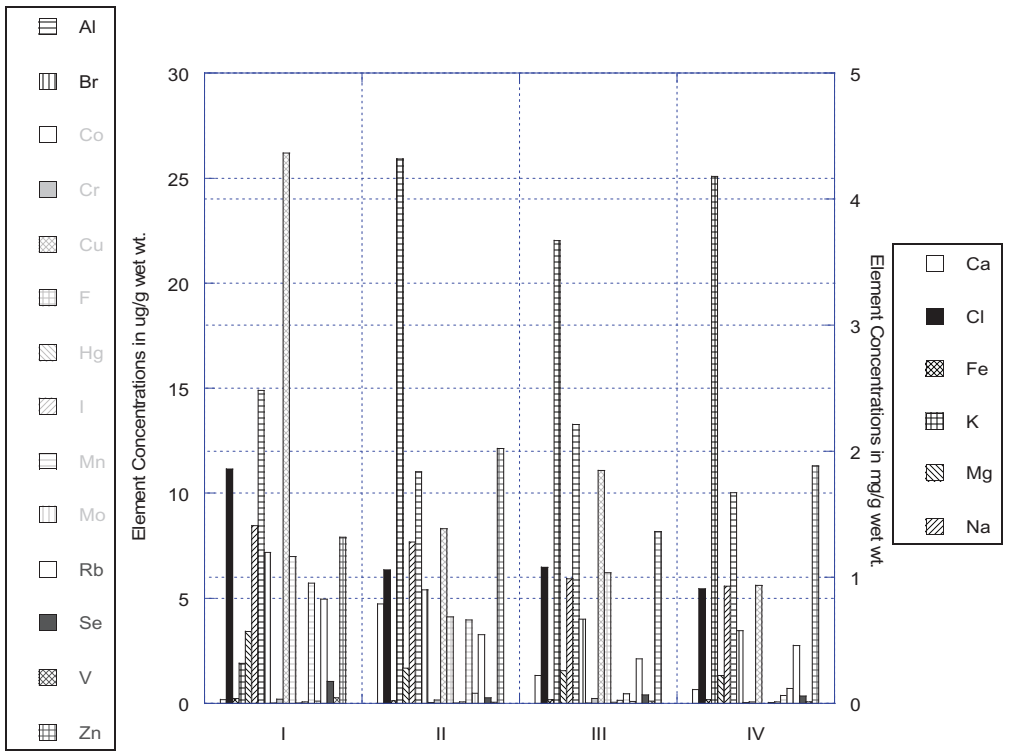

Fig. 1. Element Concentrations of Rat Cerebellum at Different Development Stages.

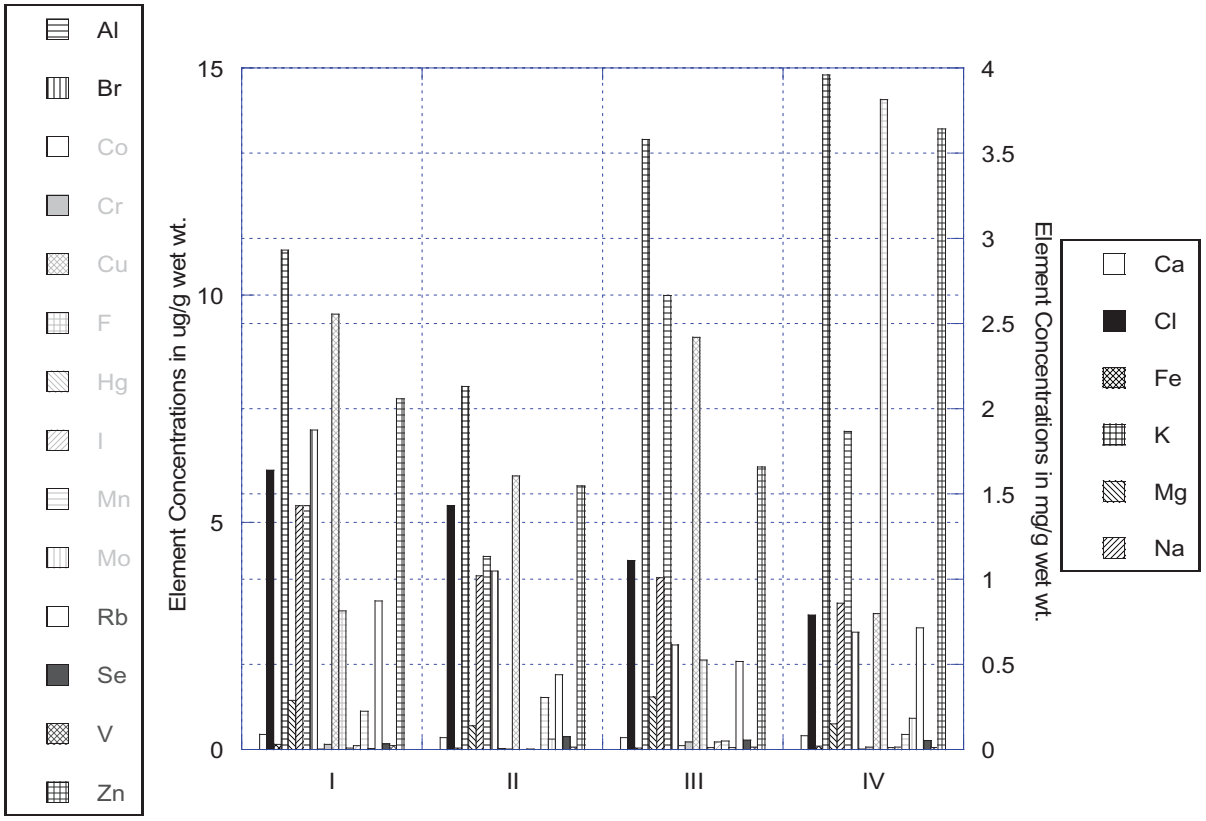

Fig. 2. Element Concentration of Rat Cerebral-Cortex at Different Developmental Stages

The cerebral cortex is responsible for all intellectual and other higher functions such as thinking, voluntary movements, language, reasoning, and perception. Figure 2 shows the elemental distributions in the cerebral cortex during postnatal development till adulthood. It can be gleaned from the results shown in Figure 2 that on average, about half of the concentrations of different elements increase with increasing age while about half of the levels of the other elements decrease as age advances toward adulthood.

Several elements in the human cerebral cortex have received more attention recently; they are described in further details in the following:

Even though the physiological role of aluminum (Al) in brain has not been identified, it is accumulated in the cerebral cortex of the rat as it matures to adulthood: presumably, this accumulation reflects dietary intake (Figure 2). Consequently, increasing brain accumulation of Al, which is known to be neurotoxic, could induce neurological problem(s) in the long term. For example, Al shows significantly higher level in the parietal cortex in AD patients compared to those in control individuals [7]. Similarly, Al accumulation in brain and other tissues, including bone, liver, and kidney, has also been shown to accompany renal failure [3].

Calcium (Ca) levels in the rat cerebral cortex do not markedly change during postnatal development (Figure 2). On the other hand, one of the pathological hallmarks of Alzheimer's disease (AD) is the accumulation of amyloid plaques between nerve cells (i.e., neurons) in different parts of human brain including cerebral cortex. Ca levels in human amyloid plaques in AD are elevated. Consistent with the latter observation is the finding that, in PSAAP mice (a mouse model of AD, which shows plaques but little neurodegeneration), Ca accumulation in their plaques is less than those in the surrounding brain tissue [6]. Consequently, age-related accumulation of Ca in this brain region could potentially serve as marker for the likelihood of developing neurodegeneration as age advances.

Chromium (Cr) shows a moderate increase in parietal cortex of AD patients compared to those in control subjects [7]. In rat cerebral cortex, Cr levels increase from day 10 to day 22/23 and thereafter decrease as adulthood is reached (Figure 2). Nevertheless, relations between brain Cr levels and neurological diseases have not been firmly established.

Copper (Cu) in amyloid plaques in AD is elevated [4]. In PSAAP mice (a mouse model of AD, which shows plaques but less neurodegeneration) Cu accumulation in their plaques is less than those in the surrounding brain tissue [6]. Consequently, increasing Cu accumulation in cerebral cortex as age advances can potentially herald the onset of neurological problems.

The magnesium (Mg) levels in rat cerebral cortex fluctuate during development and level off toward adulthood (Figure 2). Mg levels in parietal cortex and cerebellum in AD patients do not differ from those in control subjects. Taken together, these observations suggest Mg to be more predictor of neurological problems as age advances.

Manganese (Mn) shows significantly higher levels in the parietal cortex of AD patients compared to those in control subjects [7]. However, cerebellar Mn levels in AD are similar to those in control subjects [7]. Figure 2 shows that Mn levels in cerebral cortex of the rat show a general trend of decrease as its age increases. Nonetheless, whether this is a generalized trend of an essential element remains to be established.

Potassium (K) concentrations in the AD brain appear to be similar to those in control brain, especially in the regions examined [1]. K generally shows a trend of age-related increases in the rat cerebral cortex (Figure 2), perhaps reflecting increases in cellular material because intracellular K is higher than extracellular K.

Sodium (Na) levels in the rat cerebral cortex decrease with increasing age (Figure 2), reflecting the closing of the blood-brain barrier thereby excluding the entry of extracellular Na into cells. On the other hand, Na levels in every region of the AD brain are higher than corresponding levels in control brain [1]. Consequently, age-related increases in brain sodium may be considered at least a partial predictor of age-related neurological problems.

Zinc (Zn) is elevated in the amyloid plaques in AD and PSAPP mice (an AD mouse model that shows little or no neurodegeneration) only had a 29% increase of Zn in their plaques compared to the Zn in brain tissue surrounding the plaques [6]. By contrast, Zn levels in the rat cerebral cortex markedly increase between age 22/23 days and adulthood suggesting that the increase is associated with tissue growth. However, the increase in brain Zn during brain aging may be more predictive of onset and/or presence of neurological problems [6].

Hippocampus

The hippocampus is located deep within the temporal lobe and is part of the limbic system. This brain region is responsible for learning and formation of long-term memory. Figure 3 shows the elemental distributions in the rat hippocampus at different stages during postnatal development. We discuss below the elements that were emphasized in several recent reports.

Following oral exposure of rats to aluminum (Al), its retention has been noted in the hippocampus as occurs in other brain regions: rat hippocampal Al levels increase markedly after 10 days of age (Figure 3). In patients with PD, elevated Al levels have been found in several brain regions including the hippocampus, which is rich in cholinergic neurons [3]. Consequently, chronic accumulation of Al in this brain region over a life-span may lead to the development of neurodegenerative diseases such as PD because of Al is known to be neurotoxic.

Potassium (K) levels in the rat hippocampus increase from age day 10 till adulthood, likely reflecting the increase in cellular material during this developmental period (Figure 3). However, in the brain regions examined in AD, brain K remains essentially unchanged compared with corresponding levels in control subjects [1], suggesting that brain K may not be a good indicator of the likelihood of developing neurological diseases such as AD.

Rubidium (Rb) levels in rat hippocampus fluctuate between age day 10 and adulthood (Figure 3). However, Rb levels in the AD brain do not differ from those in control brain [1] suggesting that Rb levels in brain may not be a good predictor neurodegenerative diseases such as AD.

Sodium (Na) levels in the rat hippocampus decrease between ages day 10 and day 22/23 and level off at adulthood (Figure 3). However, Na levels in every part of the AD brain examined are higher than corresponding levels in the control brain [1].

Hypothalamus

The hypothalamus is located at the interior of the brain under the thalamus. It controls body temperature, emotion, thirst, hunger, appetite, digestion, and sleep. Figure 4 shows the elemental distributions in rat hypothalamus in the four developmental stages we have investigated. Even though this brain region is known to accumulate a variety of metals, their precise functional significance remains to be fully elucidated. Only few functionally important changes during development can be commented on (Figure 4).

The electrolytes in this rat brain region show fluctuating trends in the four age groups investigated, partly reflecting the closing of the blood-brain barrier. For example, sodium (Na) and chlorine (Cl) levels significant decrease in the first 22/23 days postnatal. On the other hand potassium (K) levels markedly increase between day 5 and day 10 postnatal.

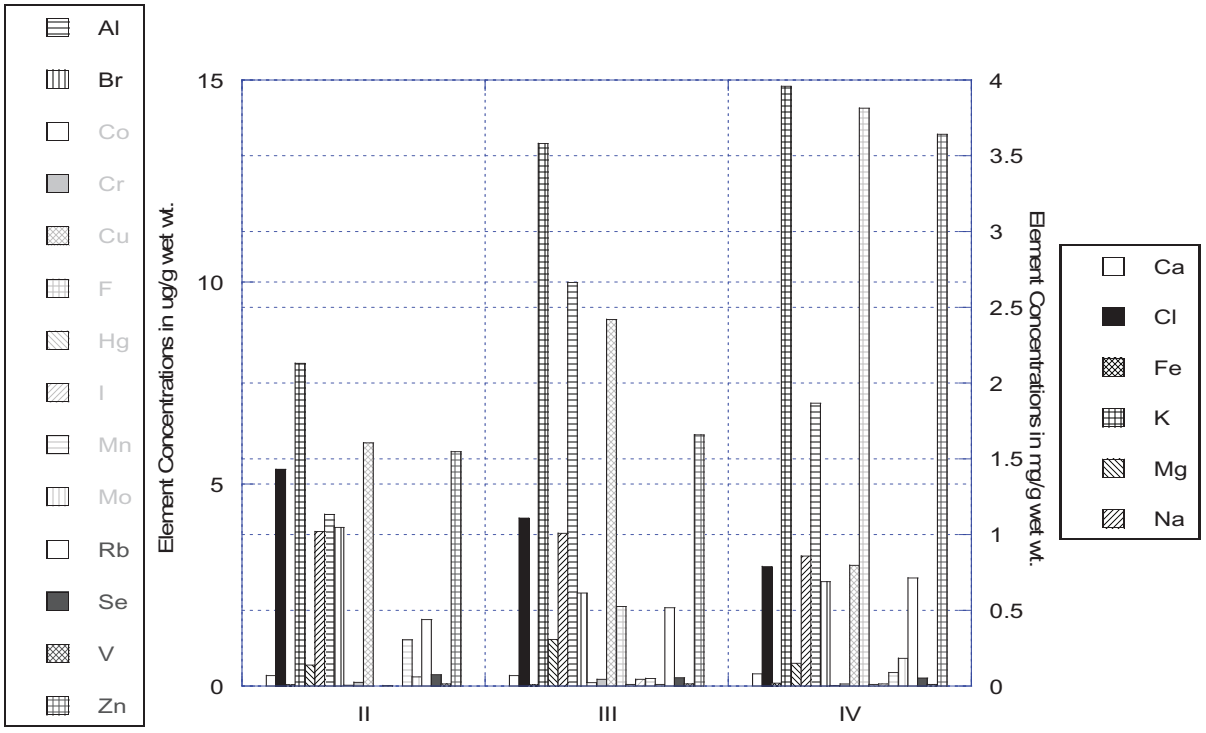

Fig. 3. Elemental Concentrations of Rat Hippocampus at Different Developmental Stages

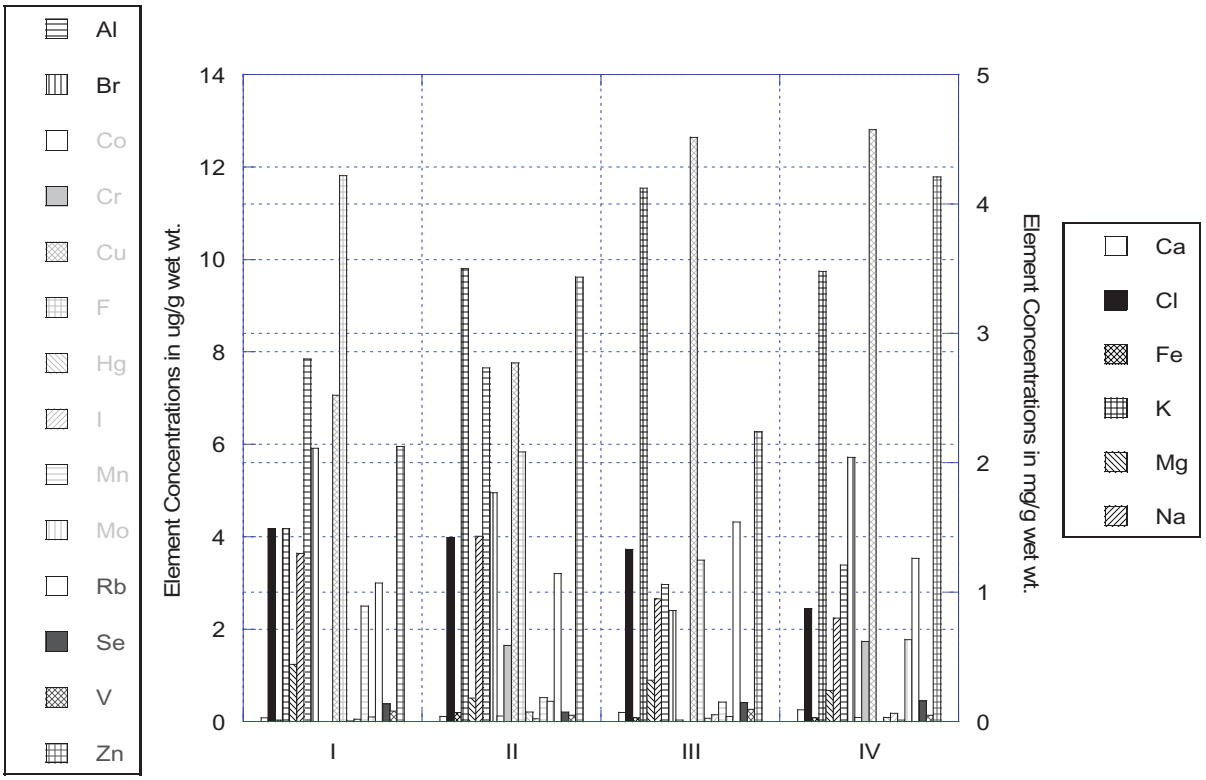

Fig. 4. Element Concentrations of Rat Hypothalamus at Different Developmental Stages

Although they show some age-related fluctuations, the essential trace elements copper (Cu), selenium (Se), and zinc (Zn) levels in the rat hypothalamus significantly increase between postnatal age 5 and adulthood (Figure 4). At present, one can only speculate about the functional significance of the postnatal increases of these essential metals in the rat hypothalamus because of the absence of relevant studies.

Mid-Brain

As its name implies, the mid-brain is located in the middle of the brain above the brain stem. The mid-brain generally acts as a relay station for both sensory and spinal cord signals and passes them on to the limbic system and the cortical areas. Figure 5 shows the elemental distributions in the rat mid-brain in the four age groups we have investigated. In the rat mid-brain, the levels of both major and trace elements studied show age-related fluctuations that differ from those in the rat cerebral cortex or hippocampus. However, the relevance of these fluctuations in the mid-brain to neurological diseases remains to be elucidated.

Pons and Medulla

Situated immediately below the mid-brain, pons and medulla constitute the brain stem which is connected to the spinal cord. Key functions of pons include motor control and sensory analysis and signal relay between the mid-brain and spinal cord. The medulla is known to be responsible for maintaining heart and breathing rates. Figure 6 shows the elemental distributions in the rat pons and medulla in the four age groups we have investigated. Most major and trace elements show age-related decreases in their levels in rat pons and medulla as adulthood approaches (Figure 6). Overall, the accumulation of metals in this brain region is not as quantitatively marked as those in the other brain regions, possibly due to the fact that this brain region is full of fiber tracts rather cell bodies. Moreover, the physiological significance of the distributions of metals in this brain region is still poorly understood.

Striatum

The striatum constitutes the sub-cortical parts of the forebrain: it includes the substantia nigra and the globus pallidus. Among its best known function is its role in the planning and modulation of movement pathways as well as roles in other cognitive functions. Figure 7 shows the elemental distributions in the rat striatum in the four age groups we have investigated. We discuss below the elements that were emphasized in several recent reports.

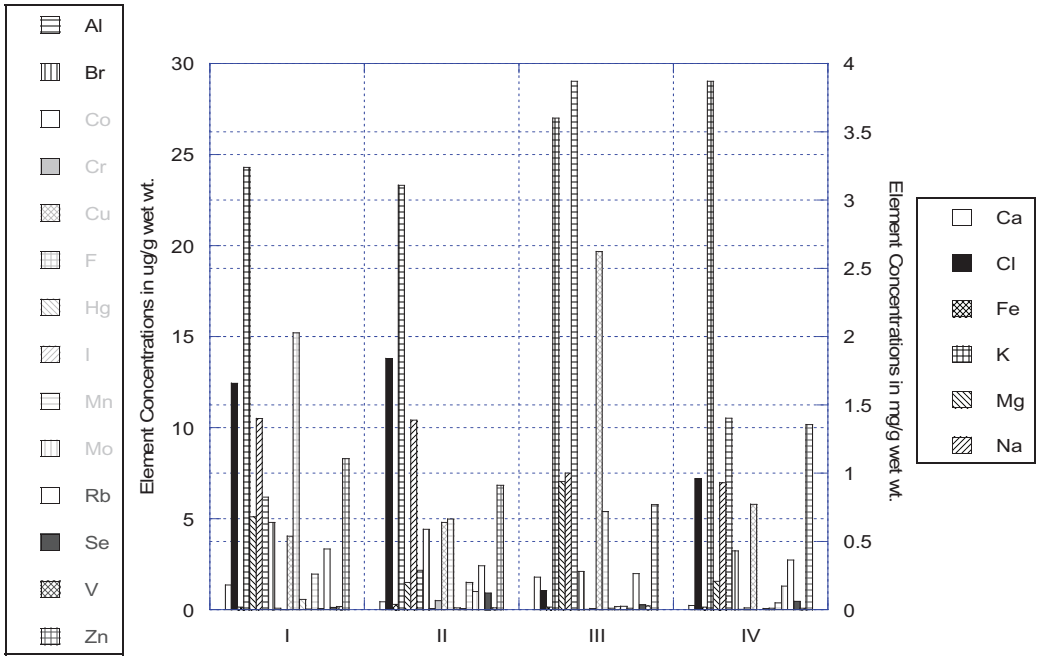

Fig. 5. Element Concentrations of Rat Mid-Brain at Different Developmental Stages

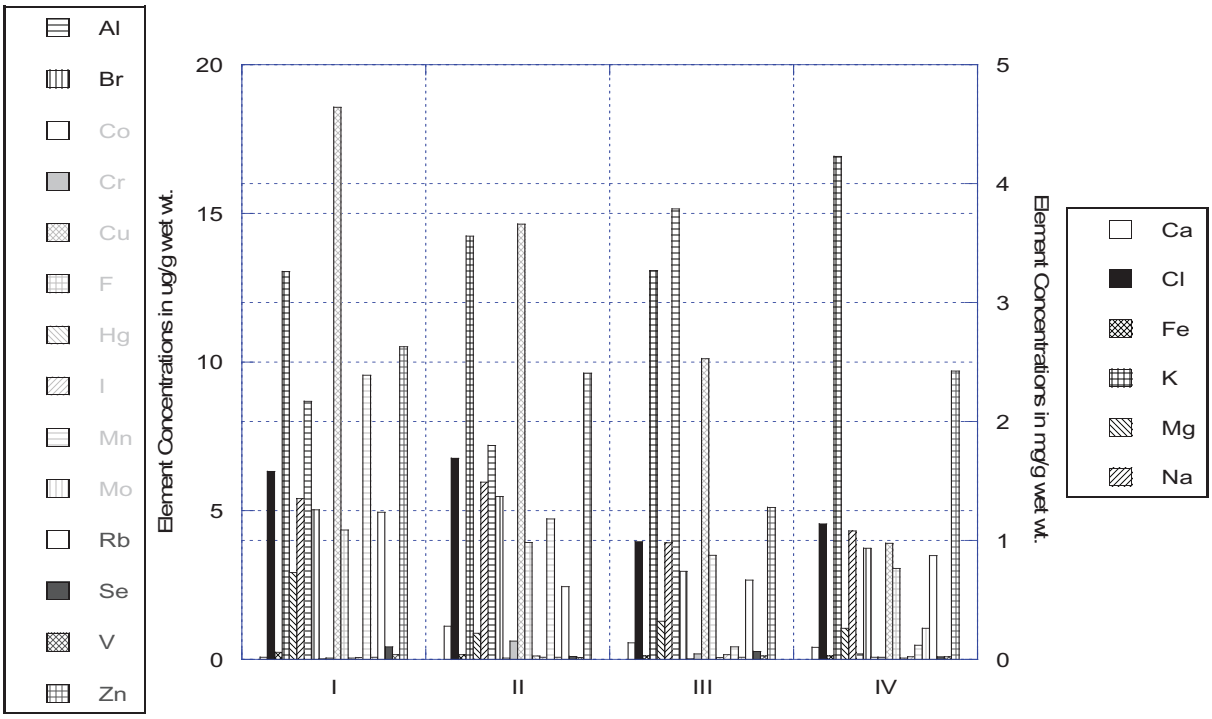

Fig. 6. Element Concentrations of Rat Pons & Medulla at Different Developmental Stages

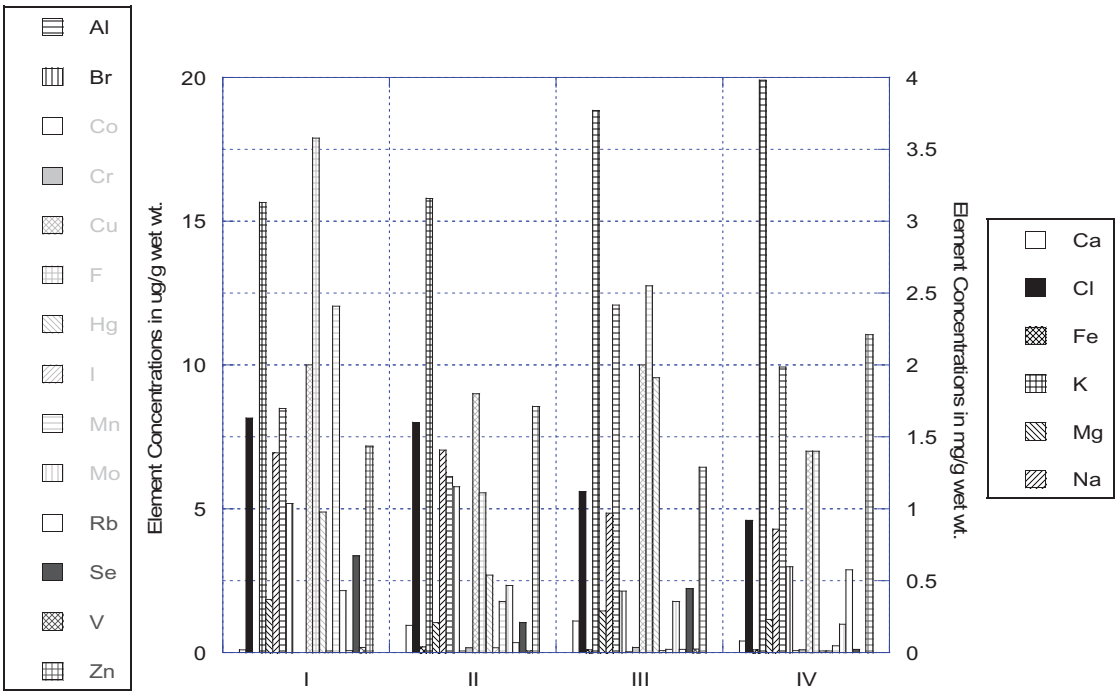

Fig. 7. Element Concentrations of the Striatum Different Developmental Stages

There is recent evidence that iron (Fe) plays a role in the consolidation of long-term memory. Brain Fe distribution overlaps with that of dopaminergic neurons. In rodent models of neurological disorders, Fe levels in the substantia nigra and basal ganglia increase. Because of those observations, increased striatal Fe has been suggested to be responsible for neuronal cell. Furthermore, Fe accumulates in brain as a function of age [19]. In rat striatum, Fe levels decrease between ages 10 days and 22/23 days and level off thereafter (Figure 7).

Brain Fe is highest in the substantia nigra, globus pallidus, red nucleus, caudate nucleus, and putamen. Increased brain Fe has been found in several neurodegenerative disorders although it has not been defined as the main cause [21]. Fe level in the substantia nigra increases in PD but total Fe in the substantia nigra zona reticulata does not change in either PD or AD [11]. Thus, these findings suggest that over accumulation of iron in striatum may lead to development of neurodegenerative diseases such as PD and AD and sources of Fe exposure could be environmental and dietary. However, it remains to be determined if the elevated Fe levels antedate injury of pigmented neurons or constitute a consequence of neuronal degeneration because the increased Fe in the substantia nigra may contribute to oxidative damage to neurons. Nevertheless, most brain Fe is stored in an inactive form bound to intracellular ferritin, which is thought to be mainly localized in microglia and oligodendroglia. Clearly, additional studies are needed to clearly delineate the pathophysiological role of Fe in neurodegenerative diseases.

Chronic manganese (Mn) toxicity in human induces signs and symptoms that closely resemble those noted in PD. There is a great deal of evidence that human striatum, globus pallidus, and substantia nigra show preferential increases in Mn and are believed to be the primary sites associated with Mn neurotoxicity. Moreover, exposure to high Mn leads to elevation of Mn levels in olfactory epithelium and olfactory bulb [14].

In every region of the brain examined, brain sodium (Na) is increased in AD compared with corresponding levels in control subjects [1]. On the other hand, Na levels in rat striatum decrease during postnatal development. Taken together, these observations suggest that high Na accumulation in brain may lead to pathological states and sources of the Na could be environmental and/or dietary in origin.

4. Conclusions

Our results strongly suggest that the metallomic distributions in various regions of the rat brain change markedly during the different stages of postnatal development. In conjunction with the reports in the literature, our findings

also suggest that brain regional metallomic distribution can be influenced by dietary intake of metals and other elements and may pathophysiological implications in several key neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. A metallomic distribution model of various regions of the rat brain can be developed based on ours experimental results and statistical analysis. However, much remains to be discovered regarding the brain functional significance of metallomic distribution during development.

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